Pedobiologia 59 (2016) 1–10

Contents lists available at ScienceDirect

Pedobiologia - Journal of Soil Ecology

journal homepage: www.elsevier.de/pedobi

Nitrification and ammonia-oxidizing bacteria shift in response to soil moisture and plant litter quality in arid soils from the Patagonian Monte

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ARTICLE INFO

Article history:
Received 20 August 2015
Received in revised form 11 November 2015
Accepted 23 November 2015

Keywords:
Nitrification
Arid soils
Ammonia-oxidizing bacteria
Ammonia-oxidizing archaea

ABSTRACT

We aimed to evaluate the effects of both plant litter quality, characteristic of sites with different histories of grazing disturbance, and soil water content on nitrification in soils from an arid ecosystem of Patagonia. To reach this goal, soil microcosms covered by plant litter of different quality and subjected to different soil water conditions were sampled at different times to analyze: (i) the nitrifying enzyme activity; (ii) the concentration of inorganic forms of nitrogen; and (iii) the abundance of bacterial (AOB) and archaeal (AOA) *amoA* genes. Soil water enhanced nitrifying activity in average 16% during the period of highest nitrification rates, and nitrate concentration in average 733% after 70 days of incubation. Microcosms amended with high litter quality showed the highest ammonium and the lowest phenolics concentrations, and higher or equal nitrification rates than microcosms amended with poor litter quality. After one week of incubation, the combination of both high litter quality and soil water significantly enhanced *amoA* gene abundance from AOB ($p < 0.05$). The AOB:AOA *amoA* genes ratio ranged from 12 to 3170. Altogether, our results suggest that high soil water and litter quality exerted positive effects over the nitrifying activity and the abundance of AOB but not AOA in these arid soils.

1. Introduction

Although scarce attention is paid to drylands compared to agricultural lands or forests, these are globally important ecosystems that cover a large area (more than 41%) of the Earth's terrestrial surface (Niemeijer et al., 2005). In arid ecosystems, with spatially and temporally discontinuous distribution of resources, water inputs are the main factor controlling biological processes (Noy-Meir, 1973). However, nitrogen is, after water, the most important nutrient controlling primary productivity in arid and semiarid lands (Belnap, 1995), and hence it is of major importance to understand the processes that regulate its abundance and dynamics in soils. Nitrification, the biological oxidation of ammonia to nitrate *via* nitrite (Haynes, 1986), is a critical process in the N cycle of terrestrial ecosystems, because it regulates the form and mobility of inorganic nitrogen in soils and its availability for plants (Austin et al., 2006; Yao et al., 2011).

The rate-limiting step in the aerobic nitrification is the oxidation of ammonia (Wong-Chong and Loehr, 1975), a reaction that is usually studied by targeting the *amoA* marker gene, which encodes the α subunit of the ammonia monooxygenase enzyme. This reaction has for long been attributed to a group of chemo-lithoautotrophic β and γ-proteobacteria named AOB (Koops et al., 2006). In the last decade, it was found that some AOA are also capable of ammonia oxidation and that archaeal genes encoding the enzymes responsible for ammonia oxidation are globally distributed (Norton and Stark, 2011). However, it is still unclear whether it is the bacteria or the archaea that have predominant roles in ammonia oxidation in soils, and although several studies indicated predominance of AOA (Adair and Schwartz, 2008; Chen et al., 2008; Leininger et al., 2006; Zhang et al., 2012), there has also been evidence of the opposite (Banning et al., 2015; Jia and Conrad, 2009; Jung et al., 2011; Wu et al., 2011).

Abbreviations: AOB, ammonia-oxidizing bacteria; AOA, ammonia-oxidizing archaea; H, site under high grazing pressure; L, site under low grazing pressure; HL, microcosms amended with plant litter from H; LL, microcosms amended with plant litter from L; CTRL, control microcosms without litter.

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http://dx.doi.org/10.1016/j.pedobi.2015.11.002
0031-4056/

Vegetation in arid and semiarid lands is composed of shrubs and grasses, which display different growth, defense, and nutrient conservation strategies, influencing the chemistry of their green and senescent tissues (Mazzarino et al., 1998). Grass litter supply a more labile C-rich substrate for decomposers than woody plant litter (Aerts and Chapin, 2000; Bosco et al., 2015; Carrera et al., 2005). In addition, because of differences in N-resorption efficiency from senescent leaves, leaf litter produced by these plant groups has different N concentration and C:N ratio (Campanella and Bertiller, 2008; Carrera et al., 2009). Concentrations of C and N in plant litter (C:N ratios) as well as the quality of C compounds influence microbial activity and litter decomposition (Carrera et al., 2009). Shrubs also present lignified stems and high concentration of other secondary metabolites (*e.g.*, phenolic compounds, including tannins), which function as defenses against UV-B damage, water stress, pathogens and herbivory (Aerts and Chapin, 2000; Mazid et al., 2011). Unlike labile C substrates from grass litter, secondary compounds are recalcitrant to microbial degradation, particularly polyphenols may also inhibit decomposition (Hättenschwiler and Vitousek, 2000; Reyes-Reyes et al., 2003).

Livestock grazing induces several changes in ecosystems through their direct and indirect impacts on vegetation, soil nutrients and on the soil conservation state. For example, grazing may change the spatial distribution of nutrients in soil through urine and feces deposition (Shand and Coutts, 2006). In infertile ecosystems with low herbivore densities, the positive effects of nutrient return from animal excreta are highly localized and may not be able to produce significant effects at the ecosystem scale (Bardgett and Wardle, 2003; Wardle et al., 2004). In addition, herbivores may change the soil bulk density through trampling (Le Roux et al., 2003). Regarding their impact on vegetation, livestock grazing reduces total plant cover and produces changes in the species composition of plant patches (Bertiller and Bisigato, 1998). One of the most conspicuous effects of long term grazing disturbance is the reduction of the cover of the most preferred plant species (perennial grasses and some deciduous shrubs) and the increase of the absolute or relative cover of most evergreen shrubs. In the Patagonian Monte, some evergreen shrubs such as *Larrea divaricata*,*Nassauvia fuegiana*, and *Junellia seriphioides* increase their relative cover, while perennial grasses such as *Nassella tenuis,* and *Poa ligularis* decrease their relative cover under heavy grazing disturbance (Bisigato and Bertiller, 1997; Bertiller and Bisigato, 1998; Larreguy et al., 2014). These changes in plant species in heavily grazed sites compared to lightly or non-grazed sites result in low quality (more recalcitrant) plant litter and in turn, influence the input of nutrients into the soil matrix (Carrera and Bertiller, 2013; Olivera et al., 2014). Moreover, grazing-induced changes in the size and composition of plant patches were associated with increments in soil erosion and degradation and low soil organic C concentration (Bertiller and Bisigato, 1998; Larreguy et al., 2014). Previous studies in the Patagonian Monte showed that plant litter with high concentration of lignin and soluble phenolics from heavily grazed sites slows down decomposition (Carrera and Bertiller, 2013; Vargas et al., 2006), has a negative effect on soil enzyme activities and microbial biomass (Olivera et al., 2014), and also affects soil bacterial diversity (Olivera et al., 2016). However, little is known about the specific effect that changes in plant litter quality produce on the soil nitrifying microbiota.

The potential influence of plant litter quality on nitrification might also vary with soil water content, through its impact on litter decomposition and nutrients release (Liu et al., 2006). Water availability controls nitrification through its effect on nitrifier accessibility to substrates, metabolism and physiology (Norton and Stark, 2011). Under dry conditions, water films in soil pores become thinner, restricting the mobility of substrates to nitrifiers (Stark and Firestone, 1995). Alternatively, nitrification also declines in soils that remain flooded for long periods, probably due to oxygen limitation (Norton and Stark, 2011). Furthermore, water availability controls microbial activity, causing physiological stress to microorganisms under very dry conditions (Schimel et al., 2007). In the Patagonian Monte, precipitations are low and may predominate during autumn and winter when, coupled with low temperatures, determine wetter conditions than in summer (soil water >10% during winter and 5–6% in summer, Coronato and Bertiller, 1997). A seasonal study in the same region showed that soil water content in combination with microbial biomass-C and total soil-N were the best predictors of soil protease activity, suggesting that dry periods with low humidity and low N-input may restrict soil proteolysis which in turn, is associated with ammonium release and N availability (Olivera et al., 2014). As a consequence, nitrification in this environment may be restricted during the dry season through low microbial activity, low proteolysis and limited accessibility of nitrifiers to substrates.

The aim of this research was to test under controlled conditions the effect of plant litter quality and soil water content on nitrification in arid soils from Patagonia. Plant litter was representative of two sites with different histories of grazing disturbance, and hence differed in C and N concentrations, C:N ratio, and concentration of labile *vs*., recalcitrant C compounds. Soil water contents tested were representative of those found in dry and wet seasons in the study area. We evaluated: (i) the nitrifying activity, (ii) the concentration of inorganic forms of N (nitrate and ammonium), and (iii) the abundance and ecological relevance of AOB and AOA in soils from an arid ecosystem. We hypothesized that both low water availability and plant litter quality slow down the nitrifying activity and decrease the abundance of the ammonia oxidizing microorganisms.

2. Material and methods

2.1. Sampling and microcosm set up

Sampling was performed at the field “La Esperanza” (42°12′S, 64°58′W), covering an area of about 6975.7 ha in NE Chubut province, within the Argentinean Patagonia. This area belongs to the southern part of the Monte Phytogeographic Province, where vegetation corresponds to a shrubland of *L. divaricata* Cav. and *Stipa* spp. (León et al., 1998). Plant cover is scarce and distributed in patches of shrubs and grasses separated by bare soil (Bisigato and Bertiller, 1997). This patchy organization of vegetation has important implications in the distribution of soil resources, in the creation of sheltered areas with favorable microclimatic conditions for seeding emergence and plant establishment, and in the protection of soils from erosion and nutrient losses (Bisigato et al., 2005). Mean annual temperature is 13.6 ± 0.7 °C (32-year average, data from Automatic Weather Station, Climatology Laboratory, CENPAT) and mean annual precipitation is 188 mm, mainly concentrated in autumn and winter (Barros and Rivero, 1982). Soils are a complex of Typic Petrocalcids-Typic Haplocalcids (del Valle, 1998; Soil Survey Staff, 1998). This ecosystem has been exposed to continuous sheep grazing since the beginning of the last century in paddocks of *ca.* 2500 ha with a single watering point (Ares et al., 2003). Under these grazing conditions, grazing pressure is higher in places nearby watering points compared to sites distantly located from them (Bisigato et al., 2005). With the conversion of “La Esperanza” into a wildlife refuge in 2003, the sheep stocking rate of the field was reduced, and after five years all sheep were removed (Bär Lamas et al., 2013). However, signs of different grazing pressures in soil and vegetation characteristics within paddocks persist nowadays. We selected two sites with